

recombination of the flanking sequences with the complementary sequences in the target chloroplast genome, and wherein said stable integration is directed into a conserved transcriptionally active ~~polycistronic~~intergenic spacer region of the chloroplast genome.

191. (Three Times Amended) A universal integration and expression vector competent for stably transforming the chloroplast genome of higher plant species which comprises an expression cassette which comprises, operably joined, a heterologous DNA sequence coding for a peptide of interest and control sequences positioned upstream from the 5' and downstream from the 3' ends of the coding sequence to provide expression of the coding sequence in the chloroplast genome of a target higher plant, and flanking each side of the expression cassette, flanking DNA sequences which originate from a plant species different from the target plant, said flanking sequences being conserved in all higher plants and complementary to the corresponding chloroplast sequences of the target plant, which flanking sequences are also competent to undergo homologous recombination with said complementary sequences of the target plant which are homologous to a spacer sequence of the target chloroplast genome, which sequence is conserved in the chloroplast genome of different plant species, whereby stable integration of the heterologous coding sequence is facilitated through homologous recombination of the flanking sequences with the homologous sequences in the target chloroplast genome into a conserved transcriptionally active ~~polycistronic~~intergenic spacer region of the chloroplast genome of the target plant.

193. (Three Times Amended) A universal integration and expression vector competent for stably transforming the chloroplast genome of higher plant species which comprises an expression cassette which comprises, operably joined, a heterologous DNA sequence coding for a peptide of interest and control sequences positioned upstream from the 5' and downstream from the 3' ends of the coding sequence to provide expression of the coding sequence in the chloroplast genome of a target higher plant, and flanking each side of the expression cassette, flanking chloroplast DNA sequences each one a portion of a synthetic spacer 2 region between the tRNA<sup>Ile</sup> and tRNA<sup>Ala</sup> genes, said chloroplast sequences being

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conserved in all higher plants and complementary to the corresponding chloroplast sequences of the target plant, which chloroplast sequences are also competent to undergo homologous recombination with said complementary sequences of the target plant which are homologous to a spacer sequence of the target chloroplast genome, which sequence is conserved in the chloroplast genome of different plant species, whereby stable integration of the heterologous coding sequence into the chloroplast genome of the target plant is facilitated through homologous recombination of the flanking sequences with the homologous sequences in a conserved transcriptionally active ~~polycistronic~~intergenic spacer region of the target chloroplast genome.

196. (Three Times Amended) A process for stably transforming higher target plant species which comprises introducing a universal integration and expression vector into the chloroplast genome of the target plant species and allowing the transformed plant to grow, the vector being competent for stably transforming the chloroplast genome of higher plant species which comprises an expression cassette which comprises, operably joined, a heterologous DNA sequence coding for a peptide of interest and control sequences positioned upstream from the 5' and downstream from the 3' ends of the coding sequence to provide expression of the coding sequence in the chloroplast genome of a target higher plant, and flanking each side of the expression cassette, flanking DNA sequences which originate from a plant species different from the target plant, said flanking sequences being conserved in all higher plants and complementary to the corresponding chloroplast sequences of the target plant, which flanking sequences are also competent to undergo homologous recombination with said complementary sequences of the target plant which are homologous to a spacer sequence of the target chloroplast genome, which sequence is conserved in the chloroplast genome of different plant species, whereby stable integration of the heterologous coding sequence into the chloroplast genome of the target plant is facilitated through homologous recombination of the flanking sequences with the homologous sequences in the target chloroplast genome, and wherein said stable integration

is directed into a conserved transcriptionally active ~~polycistronic~~intergenic spacer region of the chloroplast genome.

197. (Three Times Amended) A process for stably transforming higher target plant species which comprises introducing a universal integration and expression vector into the chloroplast genome of the target plant species and allowing the transformed plant to grow, the vector being competent for stably transforming the chloroplast genome of higher plant species which comprises an expression cassette which comprises, operably joined, a heterologous DNA sequence coding for a peptide of interest and control sequences positioned upstream from the 5' and downstream from the 3' ends of the coding sequence to provide expression of the coding sequence in the chloroplast genome of a target higher plant, and flanking each side of the expression cassette, flanking DNA sequences which originate from a plant species different from the target plant, said flanking sequences being conserved in all higher plants and complementary to the corresponding chloroplast sequences of the target plant, which flanking sequences are also competent to undergo homologous recombination with said complementary sequences of the target plant which are homologous to a spacer sequence of the target chloroplast genome, which sequence is conserved in the chloroplast genome of different plant species, whereby stable integration of the heterologous coding sequence is facilitated through homologous recombination of the flanking sequences with the homologous sequences in the target chloroplast genome into a conserved transcriptionally active ~~polycistronic~~intergenic spacer region of the chloroplast genome of the target plant.

198. (Three Times Amended) A process for stably transforming higher target plant species which comprises introducing a universal integration and expression vector into the chloroplast genome of the target plant species and allowing the transformed plant to grow, the vector being competent for stably transforming the chloroplast genome of higher plant species which comprises an expression cassette which comprises, operably joined, a heterologous DNA sequence coding for a peptide of interest and control sequences

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positioned upstream from the 5' and downstream from the 3' ends of the coding sequence to provide expression of the coding sequence including a transcription termination region in the chloroplast genome of a target higher plant, and flanking each side of the expression cassette, flanking DNA sequences which originate from a plant species different from the target plant, said flanking sequences being conserved in all higher plants and complementary to the corresponding chloroplast sequences of the target plant, which flanking sequences are also competent to undergo homologous recombination with said complementary sequences of the target plant and which are homologous to a spacer sequence of the target chloroplast genome, which sequence is conserved in the chloroplast genome of different plant species, whereby stable integration of the heterologous coding sequence ~~into the chloroplast genome of the target plant~~ is facilitated through homologous recombination of the flanking sequences with the homologous sequences ~~into~~ a conserved transcriptionally active ~~polycistronic intergenic~~ spacer region of the target chloroplast genome.

199. (Three Times Amended) A process for stably transforming higher target plant species which comprises introducing a universal integration and expression vector into the chloroplast genome of the target plant species and allowing the transformed plant to grow, the vector being competent for stably transforming the chloroplast genome of higher plant species which comprises an expression cassette which comprises, operably joined, a heterologous DNA sequence coding for a peptide of interest and control sequences positioned upstream from the 5' and downstream from the 3' ends of the coding sequence to provide expression of the coding sequence in the chloroplast genome of a target higher plant, and flanking each side of the expression cassette, flanking DNA sequences which originate from a plant species different from the target plant, said flanking sequences being conserved in all higher plants and complementary to the corresponding chloroplast sequences of the target plant, which flanking sequences are also competent to undergo homologous recombination with said complementary sequences of the target plant which

are homologous to a spacer sequence in a conserved transcriptionally active ~~polycistronic~~intergenic spacer region of the target chloroplast genome, which sequence is conserved in the chloroplast genome of different plant species, whereby stable integration of the heterologous coding sequence into the chloroplast genome of the target plant is facilitated through homologous recombination of the flanking sequences with the homologous sequences in the target chloroplast genome and the vector does not include a transposon.

**Please cancel Claims 97-99 without prejudice and without disclaimer of the subject matter contained therein.**

Please add the following new Claims 214-215:

214. (New) A universal integration and expression vector competent for stably transforming the chloroplast genome of higher plant species which comprises an expression cassette which comprises, operably joined, a heterologous DNA sequence coding for a peptide of interest and control sequences positioned upstream from the 5' and downstream from the 3' ends of the coding sequence to provide expression of the coding sequence in the chloroplast genome of a target higher plant, and flanking each side of the expression cassette, chloroplast DNA sequences which originate from a plant species different from the target plant, said chloroplast sequences being conserved in all higher plants and complementary to the corresponding chloroplast sequences of the target plant, which chloroplast sequences are also competent to undergo homologous recombination with said complementary sequences, whereby stable integration of the heterologous coding sequence into the chloroplast genome of the target plant is facilitated by said homologous recombination of the flanking sequences with the complementary sequences in the target chloroplast genome, wherein said stable integration is not directed into a transcriptionally inactive region of the chloroplast genome, said vector comprising a heterologous nucleotide sequence coding for a selectable phenotype wherein the flanking sequences comprise a portion of the intergenic spacer 2 region between the trnA and trnI genes of the chloroplast

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genome of a higher plant, which plant is the same as or different from the target higher plant, whereby double homologous recombination with the conserved spacer 2 region in the target plant chloroplast genome is facilitated.

215. (New) The vector of Claim 214 which comprises a heterologous nucleotide sequence coding for a selectable phenotype.

## REMARKS

We note with appreciation the indication of the allowabilty of Claims 4-84, 86-96, 107, 118, 119, 122, 168, 169, 172-176, 189, and 194-195. New Claim 214-21<sup>5</sup>~~6~~ have been added based on page 20 and 24 of the Specification. Claims 1 and 3-213, are now pending in the application. Claims 3, 171, 190-193, and 196-199 remain at issue in the case.

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The application has been further reviewed to correct minor typographical errors.

Applicant further acknowledges the Examiner's provisional obvious-type double-patenting rejection and, as a result, it is Applicant's continued intent to file a terminal disclaimer should those claims become patented. Further, Applicant has amended Claim 198 to clear any indefinite recitation of "into the chloroplast genome of the target plant."

Turning now to the Examiner's rejections of Claim 3, 171, 190-193, and 196-199 as containing new matter, the Applicant has amended those claims to remove the term "polycistronic" and has inserted "conserved transcriptionally active intergenic". As a result, the phrase now reads "conserved transcriptionally active intergenic spacer region" which is supported by page 6, lines 11-15; page 9, lines 15-20 and lines 31-33; page 10, lines 23-31; and page 21, lines 25-28; page 26, lines 1-11 of the Applicant's specification.

Turning now to the Examiner's rejections of Claims 3, 171, 190-192, and 196-199 as rejected under 35 U.S.C. §112, first paragraph for lacking enablement, the Applicant submits that at the time the application was filed, the specification enabled one of ordinary skill in the art to make or use the invention within the scope of the claims. Applicant respectfully requests that the Examiner consider page 27, lines 12-35 and page 28, lines 1-9 of the Applicant's specification where the methods to identify appropriate intergenic spacer regions, are taught. Specifically, at the time the application was filed, it was known to one skilled in the art that there were at least sixty transcriptionally active spacer regions within higher plant chloroplast genomes. (Sugita, M. Sugiura, M., Regulation of gene expression in chloroplasts of higher plants, Plant Molecular Biology 32: 315-326, 1996)(copy enclosed). Sugita et al. reported sixty transcriptionally active spacer regions, referred to as

transcription units, as can be seen in Table II and on page 318 of the article. Applicant submits that because the transcriptionally active spacer regions were known, one skilled in the art would be enabled to practice the Applicant's invention given the state of knowledge of spacer regions. As a result of this knowledge, one skilled in the art knew where the transcriptionally active intergenic spacer regions were located in the plastid genome. One skilled in the art could then use the method taught in the Applicant's specification at pages 24 - 28 to determine which of the sixty transcriptionally active spacer regions were conserved among plant species.

Applicant submits that detecting and isolating conserved spacer regions does not require undue experimentation, as one skilled in the art can use base pairs from genes on either side of the intergenic region as flanking sequences to identify and insert genes within that spacer region.

For the Examiner's convenience we have included copies of Nature Biotech, Vol. 19, 870-875 (2001), Ruf et al.; and Plant Journal, Vol. 19, 209-216 (1999). Sidirov et al., which supports the Applicant's assertion that a number of transcriptionally active intergenic spacer regions were known and used to insert genes in a variety of plants just after Applicant's filings. These references utilize the teachings of the Applicant by inserting tobacco spacer regions to transform a plant other than tobacco. These references however, use a transcriptionally silent spacer region, as opposed to this application which claims the use of transcriptionally active spacer regions. Consequently, while transcriptionally active intergenic spacer regions may not all be conserved, it would not be undue experimentation for one skilled in the art to determine whether a transcriptionally active intergenic spacer region is conserved among various species of plants, as was illustrated in the aforementioned references.

Applicant respectfully submits that the current amendments remove the §102(b) prior art rejection of Claim 192 by either Zoubenko et al. or Staub 1993 et al. Applicant's amendments recite the insertion of "a conserved transcriptionally active intergenic spacer

region,” which is not anticipated by either of the prior art references. Specifically, the Zoubenko article teaches insertion of the heterologous DNA in the trnB-rps12/7 intergenic region. As the Zoubenko article explains at page 3822, col. 1, the trnB-rps 12/7 intergenic region is a particular region of the chloroplast genome that is **unique** to the tobacco plastid genome. As a result, Zoubenko does not teach flanking sequences which are conserved among higher plants. The Applicant’s claims, as amended, now recite the insertion of “a conserved transcriptionally active spacer region,” thus the Zoubenko reference fails to anticipate the Applicant’s claims as amended. Prior to the Applicant’s invention there were no reports or successful attempts of stable integration of a foreign gene into a chloroplast genome of a higher plant other than tobacco. Furthermore, prior to Applicant’s discovery, a number of uncertainties surrounded the concept of inserting and expressing foreign genes in the spacer regions of higher plant chloroplast genomes. It was not known whether the foreign genes would be processed and translated correctly, and whether or not there would be proper processing signals within the plastids.

The Applicant respectfully submits that Staub 1993 fails to anticipate Claim 192 as amended. Applicant further reiterates their response to the last Office Action, which stated that the flanking regions taught in Staub 1993 are derived from tobacco plastid DNA as opposed to the Applicant’s claims, which illustrates flanking sequences derived from plant species different from the target plant. Further, nothing in the Staub 1993 reference suggests the insertion of a conserved transcriptionally active intergenic spacer region and, as a result, Staub 1993 fails to anticipate the Applicant’s Claim 192 as amended.

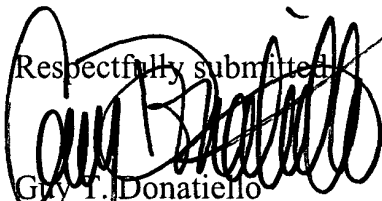
Applicant respectfully traverses the Examiner’s use of Sidirov et al. (1999) as a prior art reference. An inspection of the Applicant’s filing date reveals that the current application was filed on May 15, 1998 and, as a result, the Sidirov et al. reference of 1999 cannot serve as prior art under 35 U.S.C. §102(b). In view of the application pre-dating the Sidirov et al. reference, Applicant respectfully requests removal of Sidirov et al. as prior art.

Turning now to the potential reinstatement of the prior art rejection of Claims 3, 171

and 190-192 under 35 U.S.C. §102(b) as being anticipated by Staub et al. (1995) in light of Sidirov et al. (1999), the Applicant asserts that as a result of the amendments to the aforementioned claims, the Examiner's rejection should remain withdrawn. Applicant asserts that beyond Sidirov et al. being unavailable as prior art, there is also no support in Sidirov et al. for the insertion of the flanking sequences into conserved intergenic spacer regions of a plastid genome. Staub 1995 similarly fails to teach or suggest the use of flanking sequences conserved among higher plants. Applicant therefore requests Claims 3, 171 and 190-192 remain allowable as against Staub 1995 in view of Sidirov et al.

In light of the foregoing, allowance of the claims as amended is respectfully requested.

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